Differential Salt Sensitivity in Potato Cultivars

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Summary

As the world population continues to grow, the availability of renewable freshwater resources for agriculture will decrease, and simultaneously the area of irrigated land will increase in the attempt to satisfy the need for more food. Nowadays, irrigated land already generates one third of all food produced worldwide due to its high productivity and 20% of irrigated land is contaminated with high salt concentrations. Therefore, salinity stress is the most wide-spread environmental stress limiting crop production and the area of salt affected soils will rapidly expand in the near future for various reasons. Potato (*Solanum tuberosum*) is a major crop world-wide and the productivity of currently used cultivars is strongly reduced at high soil salt levels. Despite the agricultural importance of potato, little is known about the differences and mechanisms of salt tolerance in currently used potato cultivars.

In order to understand the physiological responses of potato plants to salt and to use that knowledge in breeding programs we treated six potato cultivars (Russet Burbank, Mona Lisa, Mozart, Mondial, Desiree and Bintje) with different salt levels in Chapter 2. Growth reduction on salt was strongest for the cultivars Mozart and Mona Lisa with a severe senescence response at 180 mM NaCl and Mozart barely survived the treatment. The cultivars Desiree and Russett Burbank were more tolerant showing no senescence after salt treatment.

In response to salt stress plants accumulate proline as a mechanism for osmotic regulation. Analysis of the expression of proline biosynthesis genes in Mozart and Desiree showed a clear reduction in proline dehydrogenase (*PDH*) expression in both cultivars and an increase in pyrroline-5-carboxylate synthetase 1 (*P5CS1*) gene expression in Desiree, but not in Mozart.

A clear difference in Na^+ homeostasis was observed between sensitive and tolerant cultivars. The salt sensitive cultivar Mozart combined low Na^+ levels in root and stem with the highest leaf Na^+ concentration of all cultivars whereas the more tolerant cultivars accumulated Na^+ in stem tissue to prevent Na^+ transport to leaves. Mozart combined a strong senescence response in leaves with a relatively high Na^+ leaf accumulation, while the potato cultivar Desiree accumulated less Na^+ in leaves and did not show a senescence response after salt treatment. In view of the premature senescence and the higher Na^+ accumulation in leaves of Mozart, we used Mozart and Desiree in a more in-depth analysis to study differences in the vacuolar Na^+ sequestration capacity between the two cultivars in Chapter 3 and 4.

In Chapter 3 we compared Mozart and Desiree for the salt induced changes in activity and expression of vacuolar H^+-pumps and vacuolar antiporters in tonoplast enriched vesicles isolated from leaves. We isolated tonoplast enriched vesicles from leaves and tested the transport characteristics of these vesicles in an ACMA fluorescence quench assay. The main conclusion from Chapter 3 is that the V_{max} of both the V-H^+-ATPase

and the V-H^+-PPase were reduced by salt in both Mozart and Desiree but the reduction was larger in Mozart as compared to Desiree. This means that Desiree is capable of generating a higher PMF across the endomembrane system as compared to Mozart. Furthermore, after salt treatment, the amount of both V-H^+-ATPase and V-H^+-PPase proteins were reduced in Mozart but not in Desiree. In addition, the Na^+/H^+ exchange system in the tolerant cultivar Desiree works more efficient as compared to the salt sensitive cultivar Mozart.

Based on the results from Chapter 3 that shows that Desiree generates a higher PMF across the endomembrane system as compared to Mozart, we decided to analyse more in detail the proton transport characteristics of tonoplasts in Chapter 4. Therefore, we isolated leaf vacuoles from control and salt treated potato- and barley cultivars contrasting in their salinity tolerance. We included vacuoles from barley since these vacuoles were easy to isolate and more stable in the patch-clamp measurements. Subsequently, we used the patch-clamp technique to measure vacuolar pump activity as substrate (PPi and ATP) induced outward proton currents.

Salt treatment showed a slightly negative effect on the $V_{\max}$ of the V-H^+-PPase in vacuoles from potato plants and a moderate increase in the PPi dependent outward currents in vacuoles of barley. Furthermore, in single vacuoles from potato and barley, salt treatment only moderately modified the ATP dependent outward currents. Therefore, we concluded that the results from the patch clamp measurements do not explain the large differences in salt sensitivity that were found before for the potato and the barley cultivars as was found with the ACMA fluorescence quench assay.

In the patch-clamp study, single vacuoles encircled by tonoplasts were used for testing the proton pump activity. In contrast, isolating tonoplast vesicles from total leaf tissue may render an assemblage of endosomal compartments in addition to tonoplasts including early- and late endosomes and the trans-Golgi network, all containing proton pumps that respond differently to salt stress. Hence, the contrasting results between the relatively strong reaction found for the proton pumps as measured with the ACMA fluorescence quench assay in Chapter 3 and the moderate reaction found for the proton pumps as measured with the patch-clamp technique in Chapter 4, may point to an important role for the endosomal compartments in salt tolerance.

In roots, K^+ retention is considered to be a trait of more salt tolerant cultivars after exposure to salt. All species tested so far, showed a salt induced K^+ efflux in root tips, the elongation zones and the mature zones of roots. Therefore, in Chapter 5, we scanned the root profile with K^+ selective vibrating probe electrodes after salt treatment to measure the K^+ efflux of potato plants contrasting in salt tolerance. In contrast to other species, we found no salt induced K^+ efflux in the mature zone of potato roots. Furthermore, salt induced K^+ efflux in the elongation zone appeared to be a poor predictor of salt tolerance in potato plants, despite strong varietal differences. In roots

of most other species, the salt induced K^+ flux showed a rapid efflux and a fast decline in the efflux in general. The salt induced K^+ efflux in roots of potato showed a slow initial increase in K^+ efflux followed by a slow K^+ efflux recovery resulting in a severe K^+ loss. This severe K^+ loss from the roots after salt treatment as compared to other species, may explain the general salt sensitivity found for commercial potato cultivars. In conclusion, this thesis illustrates the importance of Na^+ distribution throughout the plant in relation to salt tolerance in potato plants and of the endomembrane system as a way to improve our knowledge on plant salinity tolerance. The knowledge gathered in this thesis can provide new insights in breeding for salt tolerant potato cultivars.